Review

Novel Link between Inhibition of Angiogenesis and Tolerance to Vascular Stress

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The functional integrity of the vascular endothelium is an essential component required for the maintenance of vascular health, thus counteracting the onset of vascular diseases, including atherosclerosis and vascular complications of diabetes. In light of this important role, the vascular endothelium is expected to have a self-defense system. One candidate factor of such a system is vasohibin-1 (VASH1), a protein that is preferentially expressed in vascular endothelial cells (ECs). The unique features of VASH1 are its anti-angiogenic activity and ability to promote the stress tolerance and survival of ECs. This review summarizes current knowledge regarding VASH1 in terms of its roles in maintaining vascular integrity and protecting the vasculature against various forms of stress.


Key words: Endothelial cell, Vasohibin-1, HuR, SOD2, SIRT1

Introduction

The vascular endothelium, a monolayer of ECs covering the entire luminal surface of blood vessels, constitutes the interface between the circulating blood and the parenchyma of all organs. The physiological roles of the vascular endothelium are as follows: controlling the transport of various molecules across the vascular wall, regulating the immune response via the adhesion of leukocytes to the vascular wall for extravasation, manipulating the degree of vascular tonus and preventing thrombotic events. Hence, the functional integrity of the vascular endothelium is essential for the maintenance of vascular health, thereby counteracting the development of vascular diseases, including atherosclerosis and vascular complications of diabetes.

In order to play these important roles, the vascular endothelium requires a self-defense system. For example, ECs express endothelial nitric oxide synthase (eNOS) in order to generate NO, which maintains the vascular function by inhibiting vasoconstriction, platelet aggregation, leukocyte adhesion and cell proliferation via a cyclic guanosine monophosphate (cGMP)-dependent intracellular signaling pathway. Vascular endothelial growth factor (VEGF) is a representative pro-angiogenic factor, although it also acts as a survival factor for ECs by increasing the synthesis of NO by phosphorylating eNOS as well as inducing the expression of survivin. Importantly, endothelium-specific knockout of the VEGF gene results in multiple forms of vascular damage, thus indicating the essential role of endogenous VEGF synthesized by ECs in the maintenance of vascular health.

Angiogenesis, i.e., the formation of neovessels, is another physiological process associated with ECs. Angiogenesis is regulated by the local balance between its various stimulators and inhibitors, and ECs themselves may produce regulators that control angiogenesis in an auto-regulatory manner. Based on this idea, vasohibin-1 (VASH1), an angiogenesis inhibitor preferentially expressed in ECs, was recently isolated. Interestingly, subsequent analyses have revealed that VASH1 has an additional function of increasing the stress tolerance and survival of ECs. This review focuses on the significance of VASH1 in terms of its defensive role against cellular stress required for the maintenance of vascular integrity.
Isolation of Vasohibin Family Proteins by Anti-Angiogenic VASH1 and Pro-Angiogenic VASH2

Many angiogenesis regulators are extrinsic to ECs, although ECs may also produce certain angiogenesis regulators themselves. In order to search for novel angiogenesis regulators expressed in ECs, Abe and Sato stimulated ECs in culture with VEGF and performed a DNA microarray analysis to characterize VEGF-inducible genes in these cells. Their attention was focused on genes whose functions remained undefined. Recombinant proteins of such genes were made and subsequently applied to functional bioassays of angiogenesis. This attempt led to the isolation of a gene exhibiting an anti-angiogenic activity, designated vasohibin-1 (VASH1). After searching a database, a gene homologous to VASH1 was identified, designated vasohibin-2 (VASH2). The human VASH1 gene is located on chromosome 14q24.3, whereas the human VASH2 gene is located on chromosome 1q32.3. The overall homology between human VASH1 and human VASH2 is approximately 40%. Moreover, the amino acid sequences of vertebrate VASH1 and VASH2 are highly conserved. Therefore, this common ancestral gene appears to have divided into VASH1 and VASH2 during the evolution to vertebrates. The observation of this conserved amino acid sequence across species indicates the importance of the biological role of the vasohibin family.

Subsequent analyses revealed that the VASH1 protein is induced in ECs during angiogenesis in order to halt this process, whereas VASH2 is expressed in mononuclear cells mobilized from the bone marrow to stimulate angiogenesis. Accordingly, these two members of the vasohibin family are thought to be expressed in different cell types and regulate angiogenesis in a contradictory manner.

Role of VASH1 in the Maintenance and Survival of ECs

Various cellular stress causes damage to cells, resulting in premature cell senescence and ultimate cell death. When subjected to DNA damage induced by cellular stress, the cell cycle is generally arrested, leading to premature senescence. Whereas replicative senescence is the result of telomere shortening, premature cell senescence does not require such an event. Nonetheless, replicative senescent cells are more prone to DNA damage and resultant premature senescence. A premature senescent cell shows a flattened morphology and is no longer able to replicate, although it remains metabolically active and exhibits the so-called senescence-associated secretory phenotype. One of the major stressors inducing premature cell senescence is oxidative stress, which arises when the balance between the production and removal of reactive oxygen species (ROS) favors the pro-oxidation arm. Cardiovascular risk factors, such as hypercholesterolemia, hypertension and diabetes mellitus, enhance ROS generation, resulting in oxidative stress, and oxidative stress-induced damage to ECs is thought to play a very important role in the pathogenesis of vascular disease.

Atherosclerosis is accompanied by angiogenesis initiated from the vasa vasorum in the adventitia. As described by Watanabe et al., VASH1 is inducibly expressed in ECs at sites of angiogenesis. Accordingly, immunohistochemical analyses have demonstrated the presence of VASH1 in adventitial neovessels in human atherosclerotic lesions. However, in addition to their emergence on the vascular wall, VASH1 proteins are immunohistochemically detectable in arterial ECs under normal conditions. The significance of this basal expression of VASH1 was recently examined using the knockdown of VASH1, which significantly increases the number of premature senescent ECs under basal culture conditions. These senescent ECs are defective in their ability to form cell-cell junctions and are more susceptible to cell death when exposed to H2O2 or serum deprivation. In contrast, when VASH1 is overexpressed in cultured ECs, the cells become resistant to both premature cell senescence and cell death induced by exposure to H2O2 and/or serum deprivation. In addition, the blockade of VEGF signaling causes EC death, and the overexpression of VASH1 rescues ECs from this fate. These roles of VASH1 in maintaining stress tolerance have been confirmed under in vivo conditions in mice using the treatment with paraquat, which generates ROS and causes acute organ damage, including that to the lungs. In that study, compared with wild-type mice, the VASH1 (+/-) mice died in greater numbers due to acute lung injury induced by paraquat, and the treatment of these mice with an adenovirus vector encoding human VASH1 saved them from death caused by paraquat-induced acute lung injury. Therefore, the VASH1 expressed in the vascular endothelium is active in that location in order to protect the vasculature and associated organs...
The gene expression begins with the transcription of DNA for the synthesis of mRNA and ends with its translation to proteins. Post-transcriptional regulation controls the fate of mRNAs at the steps of splicing, export, stabilization and translation, and these processes are regulated by the interaction of cis-regulatory elements on the mRNA and trans-acting factors, such as RBPs and microRNAs, that bind to these elements.

HuR belongs to the embryonic lethal abnormal vision (ELAV) family of RBPs, which bind to U- and/or AU-rich elements in the 3'UTRs of their target mRNAs, and prevents their degradation and enhances translation.

There are four members of the ELAV protein family, i.e., HuB, HuC, HuD and HuR. Whereas HuB, HuC and HuD are selectively expressed in the nervous system and play roles in neuronal differentiation and plasticity, HuR is ubiquitously expressed and involved in numerous cellular responses. HuR is present predominantly in the nucleus under normal conditions. When cells are exposed to cellular stress, HuR is translocated to the cytoplasm, where it interacts with

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**Role of HuR in Stress-Induced VASH1 Synthesis in ECs**

When ECs are exposed to pro-angiogenic stimuli, such as VEGF or FGF-2, the cells gradually increase their expression of VASH1 mRNA and VASH1 protein synthesis over a 24-hour period. This induction of VASH1 is facilitated by PKCδ-mediated intracellular signaling. However, when ECs are exposed to cellular stress, they promptly increase their expression of VASH1 proteins within three hours, although this increase is not accompanied by increased transcription of VASH1 mRNA. The prompt increase in VASH1 protein synthesis observed in ECs is responsible for stress tolerance and is mediated by an RNA-binding protein (RBP), namely HuR, which acts in a post-transcriptional manner.

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**Fig. 1.** VASH1 as an anti-angiogenic and pro-survival factor of ECs.

Angiogenesis stimulators, such as VEGF, angiopoietin-1, FGF-2 and HGF, augment EC survival, whereas angiogenesis inhibitors, such as thrombospondins, angiotatin and endostatin, cause EC death. VASH1 is synthesized by ECs and exhibits the unique characteristics of not only inhibiting angiogenesis, but also promoting EC survival.
mRNAs and elicits cellular stress responses\textsuperscript{25}.

In terms of the regulation of angiogenesis, HuR has been implicated in the post-transcriptional regulation of pro-angiogenic genes, including VEGF, COX-2 and matrix metalloproteinase 9\textsuperscript{26}. VASH1 mRNA has an AU-rich element in its 3'UTR, and HuR has been shown to modulate the synthesis of VASH1 proteins by binding to this element\textsuperscript{25}. Therefore, VASH1 mRNA is now regarded as being associated with a HuR-regulated anti-angiogenic gene expected to counteract the actions of other pro-angiogenic target genes.

**SOD2 and SIRT1 Involvement in the VASH1-Mediated Stress Resistance of ECs**

**SOD2**

Oxidative stress occurs from an imbalance between the production of ROS and the capacity of antioxidant defense systems to detoxify them. The vascular wall contains enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, heme oxygenase, thioredoxin and glutathione-S-transferase, which act as members of the antioxidant defense system\textsuperscript{27}. Among these enzymes, the SOD family forms the major antioxidant defense system, with SOD existing in three isoforms: SOD1 (cytosolic Cu/Zn-SOD), as a soluble enzyme located in the cytoplasm; SOD2 (Mn-SOD), found in the mitochondrial matrix; and SOD3 (extracellular Cu/Zn-SOD), a secreted enzyme\textsuperscript{28}. These three SODs bind to the superoxide byproducts of oxidative phosphorylation and convert them to hydrogen peroxide and diatomic oxygen, thereby providing a key antioxidant function\textsuperscript{28}. SOD2, a 96-kDa homotetramer, is directed to mitochondria by a signal peptide, and, due to its localization in mitochondria, plays a critical role in protecting cells against oxidative stress\textsuperscript{29}. ECs are known to express a high level of SOD2\textsuperscript{29}, an enzyme thought to play a principal role in protecting the vascular system from oxidative stress generated by various pathophysiological processes\textsuperscript{30}.

It was recently revealed that the knockdown of VASH1 in ECs results in an increase in the ROS content and that, among various antioxidant enzymes, SOD2 is selectively decreased in expression\textsuperscript{6}. Alternatively, when VASH1 is overexpressed in ECs, there is an increase in the SOD2 expression and a decrease in the ROS content in these cells\textsuperscript{6}. Hence, SOD2 is considered to be a target of VASH1 that reduces oxidative stress in ECs.

**Sirtuin1 (SIRT1)**

Another target of VASH1 that governs the antioxidant defense of ECs is SIRT1. This enzyme is a member of the mammalian NAD\textsuperscript{+}-dependent deacetylase and ADP-ribosyltransferase family, which is orthologous to Sir2 (silencing information regulator 2)\textsuperscript{31}. Sir2 was originally isolated from yeast as a chromatin-silencing component that plays diverse biological roles in longevity, genome stability and cellular metabolism\textsuperscript{32}. Mammalian sirtuins have seven isoforms (SIRT1-7). SIRT1 is most closely related to Sir2 and is now considered to be responsible for the stress resistance of ECs. In particular, a number of reports have indicated that vascular SIRT1 protects vessels from vascular diseases, including atherosclerosis and vascular complications of diabetes\textsuperscript{34-37}.

It was recently revealed that the knockdown of VASH1 in ECs results in a decreased SIRT1 protein level and reduced activity, while VASH1 overexpression in ECs, in contrast, leads to an increased level of SIRT1 protein\textsuperscript{6}. Moreover, when the increase in SIRT1 proteins is specifically knocked down, the stress-resistance effect of VASH1 on ECs disappears\textsuperscript{6}. Therefore, SIRT1 is also regarded to be a target of VASH1 that increases the stress tolerance of ECs.

The expression of VASH1 mRNA and its post-transcriptional regulation and functional roles in ECs are summarized in **Fig. 2**.

**Pathophysiology of VASH1 in Vascular Diseases**

**Defensive Role in the Vascular Wall**

Due to the more serious paraquat-induced acute organ damage noted in \textit{VASH1 (+/-)} mice versus their wild-type littermates\textsuperscript{6}, the role of endogenous VASH1 in vascular diseases was further clarified by comparing the incidence and progression of STZ-induced diabetic vascular disease in \textit{VASH1 (+/-)} mice with that seen in their wild-type littermates\textsuperscript{38}. The results indicated that albuminuria is significantly exacerbated in diabetic \textit{VASH1 (+/-)} mice compared with that observed in their diabetic wild-type littersmtes\textsuperscript{38}. This disorder was also found to be associated with glomerular basement membrane thickening and a reduction in the slit diaphragm density. Furthermore, renal hypertrophy and the glomerular accumulation of the mesangial matrix and type IV collagen are exacerbated in diabetic \textit{VASH1 (+/-)} mice versus their diabetic wild-type littersmtes\textsuperscript{38}. Alternatively, when VASH1 is overexpressed in the lungs of \textit{VASH1 (+/-)} mice following the intra-
tracheal administration of adenoviral vectors encoding human VASH1 (AdhVASH1), paraquat-induced acute lung injury is almost completely prevented. In addition, this maneuver increases the expression of SOD2 and SIRT1 in the lungs. This protective activity of VASH1 has been further documented in murine diabetes models, as human VASH1 delivered from the liver infected with AdhVASH1 exhibits therapeutic effects on diabetic renal alterations. Such loss-of-function and gain-of-function studies have clearly demonstrated the defensive role of VASH1 in the vascular system.

The expression of VASH1 in ECs may be impaired under certain conditions. Exposure to hypoxia, inflammatory cytokines, such as TNF-α and interleukin-1, and weightlessness as well as infection with the Ebola hemorrhagic fever virus has been reported to reduce the expression of VASH1 in ECs. Such conditions may affect the maintenance of vascular health.

**Biomarker of Vascular Diseases**

As the synthesis of VASH1 in ECs is induced transcriptionally due to angiogenic stimuli or post-transcriptionally as a result of cellular stress, VASH1 proteins are detected in ECs under various pathological conditions associated with angiogenesis and cellular stress. In relation to angiogenesis, the presence of VASH1 in ECs has been documented in patients with atherosclerosis, age-related macular degeneration, diabetic retinopathy, and various cancers. As to its anti-angiogenic characteristics, an increased level of VASH1 proteins is thought to be beneficial and subsequently contribute to a better prognosis. However, contrary to expectations, an elevated expression of VASH1 has been shown to rather predict a worse clinical outcome in many, but not all, clinical studies. In fact, the situation is not that simple, as the synthesis of this angiogenesis inhibitor in ECs is augmented by angiogenic stimuli associated with cancer. Accordingly, the expression of VASH1 is more significantly elevated in patients with cancers with higher angiogenic potential, and this may be one reason for the worse prognosis of patients with an elevated VASH1 expression.

A similar situation has been documented in cases of disease associated with augmented cellular stress, namely, chronic renal disease (CKD). Importantly, one study determined the concentrations of VASH1 in CKD patients using ELISA; the results revealed that the plasma levels of VASH1 positively correlate with
VASH1 was recently isolated as a negative-feedback regulator of angiogenesis synthesized by ECs. Interestingly, this angiogenesis inhibitor increases stress tolerance of ECs and promotes their survival (Fig. 3). Elucidating the precise mechanisms accounting for the unique activities of VASH1 awaits the isolation of the vascular stress tolerance regulator VASH1. 

Fig. 3. VASH1 prevents both pathological angiogenesis and age-related vascular diseases. DNA damage causes various diseases, including cancer and age-related vascular diseases. The VASH1 expressed in ECs prevents these diseases via the inhibition of angiogenesis and tolerance to vascular stress.

Concluding Remarks

This review summarized a novel link between angiogenesis inhibition and vascular stress tolerance. The inhibition of angiogenesis prevents various diseases accompanied by pathological angiogenesis, whereas vascular stress tolerance is important for the maintenance of vascular integrity and prevention of age-related vascular diseases. As angiogenesis inhibitors generally cause endothelial cell death, these factors are not assumed to be involved in vascular stress tolerance. VASH1 was recently isolated as a negative-feedback regulator of angiogenesis synthesized by ECs. Interestingly, this angiogenesis inhibitor increases stress tolerance of ECs and promotes their survival (Fig. 3). Elucidating the precise mechanisms accounting for the unique activities of VASH1 awaits the isolation of the vascular stress tolerance regulator VASH1.

Conflicts of Interest

None.

References

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